1. A combination of epitopes of the tau protein which are specifically occurring in a phosphorylated state in tau protein from Alzheimer paired helical filaments, said combination including the phosporylatable serine residues 46, 199, 202, 235, 262, 293, 324, 356, 396, 404 and/or 422 and/or the phosphorylatable threonine residues 50, 69, 111, 153, 175, 181, 205, 212, 217 and/or 231, with the proviso that said combination is not the combination Ser 202, Ser 235, Ser 404, Thr 205.

2. The combination according to claim 1 which contains an epitope comprising the amino acid sequence KESPLQ, YSSPGSP, PGSPGT, YSSPGSPGTPGS, PKSPSS, YKSPVVS, GDTSPRH, MVDSPQL; PLQTPTE, LKESPLQTPTED, AKSTPTA, IGDTPSL, KIATPRGA, PAKTPPA, APKTPPS, PAKTPPAPKTPPS, SPGTPGS, RSRTPSL, SLPTPPT, RSRTPSLPTPPT, VVRTPPK, VVRTPPKSPSSA, KIGSTENLK, KCGSKDNIK, KCGSLGNIH, or KIGSLDNITH.

3. Use of a protein kinase for specifically converting tau protein to Alzheimer tau protein by phosphorylation of the amino acid motif ser-pro or thr-pro, said protein kinase having the following biochemical properties:
   (a) It phosporylates ser-pro and thr-pro motifs in tau protein;
   (b) it has an M_r of 42 kD;
   (c) it is activated by ATP and has a K_m of 1.5 mM;
   (d) it is activated by tyrosine phosphorylation;
   (e) it is recognized by an anti-MAP kinase antibody; and
   (f) it is deactivated by phosphatase PP2a.

4. Use according to claim 3, wherein said protein kinase is obtainable by
   (a) homogenizing porcine brain in 10 mM Tris-HCl, pH 7.2, 5 mM EGTA, 2 mM DTT and a cocktail of protease inhibitors (leupeptin, aprotinin, pepstatin A, α2-macroglobulin, PMSF);

(b) centrifugating the homogenate at 100,000 x g for 30 minutes at 4°C;
(c) removing the supernatant after centrifugation;
(d) precipitating the crude protein by ammonium sulfate precipitation;
(e) desalting the crude preparation by gel filtration;
(f) activating the crude enzyme by incubation in activation buffer;
(g) further purifying the crude preparation by ion exchange chromatography; and
(h) identifying the enzyme by Western blotting.

5. A protein kinase which is capable of specifically converting tau protein to Alzheimer tau protein by phosphorylating IGS and/or CGS motifs (Serines 262, 293, 324, 356) in the repeat region of tau protein.

6. The protein kinase according to claim 5 which is obtainable by carrying out the following procedures:
(A) subjecting mammalian brain extract to ion exchange chromatography on Mono Q (Pharmacia);
(B) testing the fractions eluted for phosphorylation of tau protein and influence on binding to microtubules;
(C) further purifying the active fractions by gel chromatography;
(D) subjecting the fraction eluting at about 35 kDal to ion exchange chromatography on mono Q; and
(E) collecting the major peak eluting between 200 and 250 mM NaCl;
and has the following characteristics:
(a) it binds to mono Q but not to Mono S;
(b) it has an acidic pI;
(c) it shows a major band (>95%) at 35 kDal and a minor band (<5%) at 41 kDal on silver-stained gels;
(d) it incorporates a phosphate amount of 3.2 Pi into htau34, as described in Fig. 19 and defined in Neuron 3 (1989), 519-526, 3.4 Pi into htau40, as described in

Fig. 18 and 19 and defined in Neuron 3 (1989), 519-526, 3.3 Pi into htau23, as defined in Neuron 3 (1989), 519-526 and 2.8 Pi into mutant htau23 (Ser262 → Ala); and

(e) it phosphorylates serine residues 262, 293, 324 and 356 of tau protein;

7. The protein kinase according to claim 5 which is obtainable by carrying out the following steps:

(A) preparation of high spin supernatant of extract from mammalian brain;

(B) subjecting the brain extract to chromatography on ion exchange Q-Sepharose (Pharmacia);

(C) testing the fractions and flowthrough for phosphorylation of tau protein and influence on binding to microtubules;

(D) chromatography of flowthrough on S-Sepharose, wherein the kinase activity elutes at 250 mM NaCl;

(E) chromatography on heparin agarose, wherein the kinase activity elutes at 250 mM NaCl;

(F) gel filtration, wherein the kinase activity elutes at 70 kDal; and

(G) chromatography on Mono Q, wherein the kinase activity elutes at 150 mM NaCl;

and has the following characteristics:

(a) it does not bind to Q-Sepharose but to S-Sepharose;

(b) it has an alkaline pI;

(c) it shows a major band around 70 kDal on SDS gels;

(d) it incorporates 3-4 phosphates into htau34, as described in Fig. 19 and defined in Neuron 3 (1989), p. 519-526, htau40, as described in Fig. 18 and 19 and defined in Neuron 3 (1989), p. 519-526, htau23, as defined in Neuron 3 (1989), p. 519-526, and the construct K19 (i.e., the four-repeat microtubule binding region);

(e) it does not phosphorylate a mutant of K19 where Ser 262, 293, 324, and 356 are mutated into Ala; and

(f) it phosphorylates Ser 262, 293, 324, and 356 or tau protein.

8. The protein kinase according to claim 5, which is a 70 kDal kinase and phosphorylates the two IGS motifs and the two CGS motifs of tau protein (Serines 262, 293, 324, 356) and may be obtained as follows:

(A) preparation of high spin supernatant of brain extract;

(B) chromatography on Q-Sepharose;

(C) chromatography of flowthrough on S-Sepharose, wherein the kinase activity elutes at 250 mM NaCl:

(D) chromatography on heparin agarose, wherein the kinase activity elutes at 250 mM NaCl;

(E) gel filtration, wherein the kinase activity elutes at 70 kDal;

(F) chromatography on Mono Q, wherein the kinase activity elutes at 150 mM NaCl.

9. Use of a protein kinase which specifically phosphorylates serines 46, 199, 202, 235, 262, 293, 324, 356, 396, 404, 422, and/or threonines 50, 69, 111, 153, 175, 181, 205, 212, 217, 231 of the tau protein for specifically converting tau protein to Alzheimer's tau protein.

10. Use according to claim 3, 4 or 9, wherein said protein kinase is glycogen synthase kinase-3 (isoform α, 51 kD and/or β, 45 kD) or cdk2-cyclin A (33 kD) or MAP kinase.

11. Use according to any one of claims 3, 4, 9 or 10, wherein said protein kinase is a protein kinase from human brain, porcine brain, or another source.

12. A pharmaceutical composition containing a specific inhibitor for the protein kinase as defined in any one of claims 3 to 11, optionally in combination with a pharmaceutically acceptable carrier and/or diluent for use in the treatment of Alzheimer's disease.

13. The pharmaceutical composition according to claim 12 which contains as the specific inhibitor a combination of oligo- or polypeptides comprising an epitope according to claim 1 or 2.

14. An antibody which specifically recognizes an epitope contained in the combination according to claim 1 or 2.

15. An antibody which specifically recognizes the protein kinase according to any one of claims 5 to 8.

16. The antibody according to claim 14 or 15 which is a monoclonal antibody.

17. A diagnostic composition for the detection and/or monitoring of Alzheimer's disease comprising:
- a combination of epitopes according to claim 1 or 2;
- a kinase as defined in any one of claims 3 to 11;
- an antibody according to claim 14 or 16; and/or
- an antibody according to claim 15 or 16.

18. A method for the <u>in vitro</u> diagnosis of the onset of Alzheimer disease comprising assaying a cerebrospinal fluid isolate of a patient or carrying out a biopsy of nerve tissue and testing said tissue for the presence of a phosphorylated serine residue in position 262 of tau protein.

19. A method for the <u>in vitro</u> diagnosis and/or monitoring of Alzheimer's disease comprising assaying a cerebrospinal fluid isolate of a patient or carrying out a biopsy of nerve tissue and testing said tissue
- for the presence of a phosphorylated Alzheimer tau protein containing a combination of epitopes according to claim 1 or 2;
- for the presence of a protein kinase as defined in any one of claims 3 to 11; or

- for the presence of phosphatases PP2a, PP1 and/or calcineurin.

20. The method according to claim 18 or 19, wherein the Alzheimer tau protein and the phosphorylation of serine residue 262 of tau protein, respectively, are detected by using an antibody according to claim 14 or 15.

21. The method according to claim 18 or 19, wherein the protein kinase is detected by using an oligo or polypeptide comprising an epitope contained in the combination according to claim 1 or 2 and/or by using an antibody as defined in claim 16.

22. A method for the in vitro conversion of the tau protein into Alzheimer tau protein wherein normal tau protein is treated with a protein kinase as defined in any one of claims 3 to 11 under conditions which allow the phosphorylation of said normal tau protein.

23. Use of an epitope contained in the combination according to claim 1 or 2 for the generation of Alzheimer tau protein specific antibodies or antibodies to a tau protein specific for the onset of Alzheimer disease.

24. A pharmaceutical composition for use in the treatment or prevention of Alzheimer's disease comprising an inhibitor of the formation of Alzheimer paired helical filaments from tau protein dimers.

25. An in vitro method for testing drugs effective in dissolving Alzheimer paired helical filaments comprising the following steps:

(a) allowing the formation of Alzheimer paired helical filaments from polypeptides comprising tau derived sequences under appropriate conditions;

(b) incubating the Alzheimer paired helical filaments with the drug to be tested; and

(c) examining the result of the incubation of step (b) with respect to the dissolution of the Alzheimer-like paired helical filaments.

26. The method according to claim 25, wherein the conditions of step (a) comprise an environment of 0.3 to 0.5 M Tris-HCl and pH 5.0 to 5.5 without additional salts.

27. An <u>in vitro</u> method for testing drugs effective in the prevention or reduction of the formation of Alzheimer paired helical filaments comprising the following steps:

(a) incubating the drug to be testing with polypeptides comprising tau-derived sequences under conditions which allow the formation of Alzheimer paired helical filaments in the absence of said drug; and

(b) examining the result of the incubation of step (a) with respect to the presence or absence of Alzheimer paired helical filaments in the incubation mixture.

28. The method according to any one of claims 25 to 27 wherein said polypeptides comprise essentially the repeats from the C-terminal part of the protein only.

29. The method according to any one of claims 25 to 28 wherein said polypeptides are K11 and/or K12.

30. A method for testing drugs effective in dissolving Alzheimer paired helical filaments comprising the following steps:

(a) introducing a functional gene encoding a MAP kinase under the control of suitable regulatory regions into a cell expressing or overexpressing tau protein;

(b) allowing the formation of phosphorylated tau protein and of Alzheimer paired helical filaments;

(c) isolating said Alzheimer paired helical filaments;

(d) applying the drug to be tested to said paired helical filaments under appropriate conditions, and

(e) examining the effect of said drug on said paired helical filaments.

31. The method according to claim 31, wherein said cell expressing tau protein is a neuroblastoma, chromocytoma or primary nerve cell.

32. Pharmaceutical composition for the treatment of Alzheimer disease comprising a PP2a and/or PP1 and/or calcineurin phosphatase as the active or one of the active ingredients.